

Introduction

Hypertrophic cardiomyopathy, an abnormal thickened heart muscle with fibers that have become disarrayed, is one class of heart disease. However, it is not clear if the abnormally thickened muscle tissue is due to expanded cell size or by an increased number of heart cells in the tissue. Previous studies have shown that overexpression and mutation of heart proteins, including, Actin Capping Protein, cause severe disruption of fibers and leads to hypertrophy. To better understand the abnormal arrangement of muscle fibers in hypertrophy at the cellular level, the heart muscle cells (cardiomyocytes) of mice with mutation of Capping Protein should be isolated and cultured to allow scrutiny of the cell shape, size and contacts with other cells.

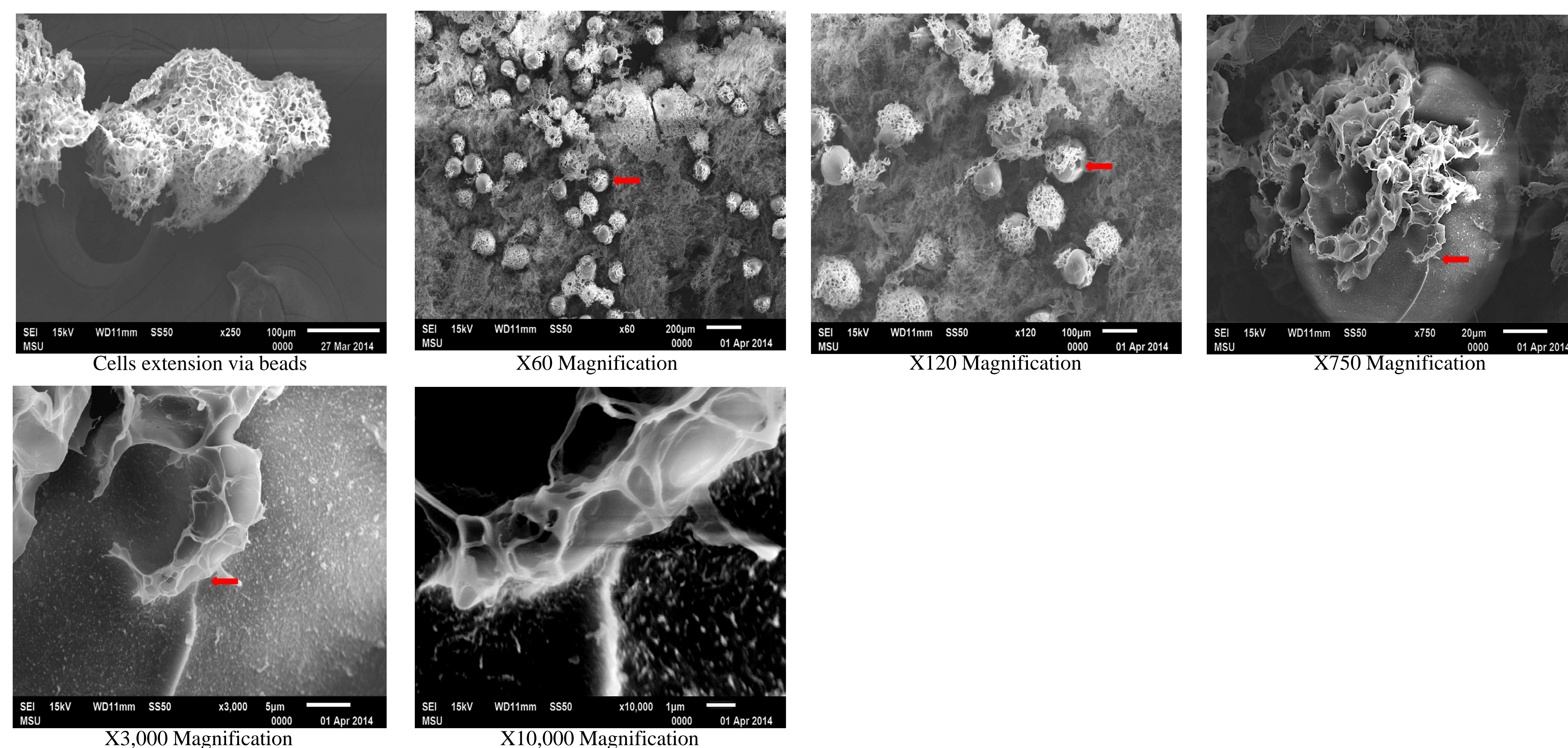
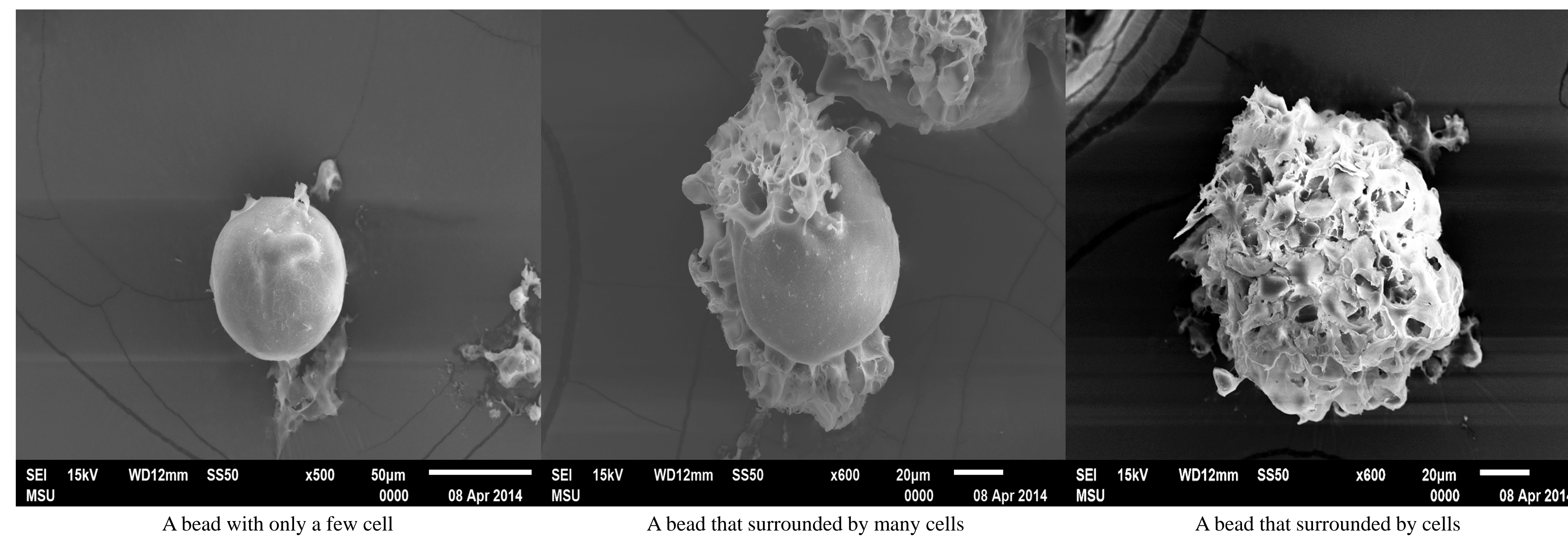
Current Study

In previous studies, the hearts were examined for changes in size and shape. However, the individual cardiomyocytes have not been analyzed.

In initial studies to acquire the necessary cell culture skill, we cultured Human Embryonic Kidney (HEK) cells onto collagen coated dextran microcarrier beads (small beads that provide an attractive surface for cell growth and division), and prepared them for direct visualization using the powerful technique of Scanning Electron Microscopy (SEM). This experiment was a “first attempt” to learn how to manipulate efficiently and prepare cells for SEM. Under SEM, we could observe cells’ size, shape, and adhesive properties with other cells. Successful HEK cell isolation, culturing and examination via SEM provide techniques and the guideline for the next experiment, cardiomyocyte examination.

Images of cells that attached to the dextran microcarrier beads

Under Scanning Electron Microscopy



Procedure

1. Human Embryonic Kidney Cells were obtained aseptically from Dr. Goellner’s lab, cultured and incubated at 37° C for four days in the presence of 5% CO₂.
2. In a six-well cell culture plate, 2mL of Dulbecco’s Modified Eagle Medium (DMEM) was added in each well.
3. Varying concentration of beads, 0.2 mg/µL, 0.33 mg/µL, 1.0 mg/µL, 2.0 mg/µL, 5.0 mg/µL, and 10 mg/µL in Phosphate Buffered Saline (PBS) solution were placed in the six wells, respectively.
4. HEK cells were isolated, and two drops of cell suspensions were added in each well.
5. Cells were incubated at 37° C for four days in the presence of 5% CO₂.
6. The cells that attached to the microcarrier beads were examined under light microscopy.
7. DMEM was removed from each well, and 2mL of 2.5% glutaraldehyde was added in each well.
8. The plate was placed in a 3-5° C fridge for three days.
9. Glutaraldehyde 2.5% was removed from each well and washed with PBS.
10. The cells were frozen in liquid nitrogen
11. The cells were freeze dried, sputter coated with gold, and visualized using a JOEL JSM 6510LV/LGS Scanning Electron Microscope.
12. Digital images were captured and analyzed.

Discussion

1. Successful HEK cell culturing onto dextran microcarrier beads showed feasibility for culturing mouse embryonic cardiomyocytes onto beads. However, we identified that different beads concentration did not affect cells growth or degree of attachment of cells.
2. Cell preparation technique to visualize for SEM was obtained, and can be used for cardiomyocytes preparation.
3. Comparing beads with no cells to beads with cells attached give us an idea what to look at when we examine cardiomyocytes.
4. Different magnifications show deliberate cell morphology.
5. The HEK cells had a network of ridges that appeared to be membrane folds. Further work is necessary to determine whether or not the ridges are preparation artifacts.

Future Studies

1. Trial of different technique for cell preparation.
2. Establishing primary cultures of mouse embryonic cardiomyocytes from both wild type and transgenic mice with altered actin capping protein.
3. Examination of cardiomyocytes attachment to the dextran microcarrier beads.
4. Examination of individual cardiomyocyte’s shape, size and juxtaposition to other cardiomyocytes.

Acknowledgements

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References

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